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Human Monoclonal Antibody to Hepatitis C Virus E1 Glycoprotein That Blocks Virus Attachment and Viral Infectivity

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Human antibodies elicited in response to hepatitis C virus (HCV) infection are anticipated to react with the native conformation of the viral envelope structure. Isolation of these antibodies as human monoclonal antibodies that block virus binding and entry will be useful in providing potential therapeutic reagents and for vaccine development. H-111, an antibody to HCV envelope 1 protein (E1) that maps to the YEVRNVSGVYH sequence and is located near the N terminus of E1 and is able to immunoprecipitate E1E2 heterodimers, is described. Binding of H-111 to HCV E1 genotypes 1a, 1b, 2b, and 3a indicates that the H-111 epitope is highly conserved. Sequence analysis of antibody V regions showed evidence of somatic and affinity maturation of H-111. Finally, H-111 blocks HCV-like particle binding to and HCV virion infection of target cells, suggesting the involvement of this epitope in virus binding and entry.

Infection with *Hepatitis C virus* (HCV), a member of the family *Flaviviridae*, leads to chronic liver disease in the majority of cases and progresses to liver failure and hepatocellular carcinoma in some cases (13). The virion is composed of a viral genome and a capsid protein that are encased in a lipid bilayer coated with the envelope proteins E1 and E2 (reviewed in reference 7). Since there is no effective vaccine and current therapy has significant limitations, information on the early events of infection, virus binding and entry, is needed for vaccine and therapeutic drug development. Although HCV is found partly in association with lipoproteins in the plasma of infected patients, suggesting that virus entry is possible via the low-density lipoprotein receptor (1, 14), the first step of infection with a virus is usually the interaction of the virus envelope glycoproteins and specific cell surface receptors. This report describes H-111, a human monoclonal antibody (HMAb) to an epitope located on the N-terminal end of E1 that blocks baculovirus-expressed HCV-like particle (HCV-LP) (2) binding to and HCV virion infection of susceptible target cells (16).

Generation of an E1 human monoclonal antibody. Antibodies elicited in response to viral infection are anticipated to react with the native conformation of the viral envelope structure and therefore are likely to be useful in studies of virus binding and entry. Fourteen plasma samples from blood donors who tested seropositive for serum antibodies to HCV were obtained and screened for antibodies to E1 antigen. In this study, a donor infected with HCV genotype 1b with the highest titer of antibody to E1 was used as the source of peripheral B cells for hybridoma isolation as previously described (15). Recombinant E1 protein expressed in HEK293 cells was used as the target antigen. DNA encoding the E1 protein was prepared from HCV RNA extracted from HCV genotype 1b-positive serum by reverse transcription (RT)-PCR with *Pfu Taq* polymerase (Stratagene, La Jolla, Calif.) and HCV-specific oligonucleotide primers (forward, 5′-AGATCT TATGAAGTGCGCAACGTGTCCGGG; reverse 5′-CTGC AGCTTAGCCCAGTTCCCTGCCAT) that contained flanking BglII or PstI restriction sites (underlined). Amplified DNA fragments were subsequently ligated into the pDisplay vector (Invitrogen, San Diego, Calif.) in frame with hemagglutinin (HA) and c-*myc* as tags. The external domain of human CD4 (amino acids 1 to 371) was amplified from peripheral blood lymphocyte cDNA and cloned into the same vector described above and served as a negative control for antibody generation. One hybridoma, designated H-111, which produced HMAb with reactivity to the E1 protein as determined by an immunofluorescence assay (IFA) was generated (11). Monoclonality was confirmed by sequencing of the immunoglobulin G (IgG) genes isolated from 10 individual cell clones derived from the hybridoma. The cell line produced IgG_1 antibody with a κ light chain and secreted approximately 80 μg of human IgG per ml in spent culture supernatant.

To determine the extent of sequence conservation among different HCV genotypes, H-111 was tested with E1 proteins representing genotypes 1a, 1b, 2a, 2b, 3a, and 4a from 19 different sources of HCV-infected sera (Table 1). Recombinant E1 plasmids (constructed in a manner similar to that for the HCV 1b pDisplay plasmid used in antibody generation as described above) were transfected into the HEK293 cells by using PolyFect reagent (QIAGEN, Valencia, Calif.) according to the manufacturer's instructions. The presence of expressed protein was verified with the HA MAb by Western blotting

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TABLE 1. H-111 reactivity to HCV E1 protein isolates from multiple genotypes[a]

Genotype	No. of isolates	Test result for antibody		
		Anti-HA (positive control)	H-111 (HMAb)	anti-CMV (negative control)
HCV 1b	1	+++	+++	−
HCV 1a	3	+++	+++	−
HCV 2b	3	+++	+++	−
HCV 2a	5	+++	−	−
HCV 3a	5	+++	++	−
HCV 4a	2	+++	−	−

[a] HEK293 cells were transfected with HCV E1 constructs comprising E1 genes cloned from the indicated HCV genotypes and analyzed by an immunofluorescence assay (IFA). The transfected cells prepared 24 h posttransfection were fixed onto slides with acetone and stained with rat monoclonal antibody to HA at 2 μg/ml, H-111 at 5 μg/ml, or R04, a negative control antibody, at 5 μg/ml. Slides were counterstained with a 0.001% solution of Evan's blue counterstain, and bound antibody was detected with fluorescein isothiocyanate (FITC)-conjugated goat anti-human or anti-rat IgG. +++, strong; ++, moderate; −, no reactivity.

(data not shown) (11). The reactivity of H-111 with the E1 proteins was assessed by IFA (10) (Table 1) and confirmed by enzyme-linked immunosorbent assay (ELISA) (data not shown). As shown in Table 1, which presents data for a total of 19 different E1 proteins, H-111 reacted with the E1 derived from the virus of the B-cell donor from which this antibody was generated and with an additional 11 E1 proteins from virus isolates of genotypes 1a, 1b, 2b, and 3a. H-111 was nonreactive with genotype 2a E1 proteins from five different sources of virus (two independent clones each), suggesting that the H-111 epitope might be mutated in genotype 2a. H-111 was also nonreactive with E1 proteins of genotype 4a (two sources, two clones each). All E1 clones from different sources were confirmed by sequencing, and representatives from each genotype were compared with known corresponding sequences from GenBank (Fig. 1).

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                      192                 211
1b    E1-107          YEVRNVSGVY HVTNDCSNSS
1b    AAK95628        .........I ..........
1a    E1-462          .Q...ST.L. ......P...
1a    E1-463          .......... ..........
1a    AAK27064        .........L ..........
1a    AAA67836        .......... ..........
2b    E1-472          V....I..I. .A......N.
2b    AAP55693        .......SS. Y.........
2b    AAA45542        .....T.... ..........
2a    E1-608          A..K.I.NS. M.....A.D.
2a    E1-611          .......TT. ......S...
2a    AAF01178        .......TG. ......T...
2a    AAA45561        .Q....TNS. ..........
3a    E1-621          L.W..T..L. VL....P...
3a    BAA03255        .......... ..........
3a    AAA45568        .......... ..........
4a    E1-625          C-Y.....I. ......P...
4a    E1-626          VN........ .I........
4a    CAA72338        VNY....... ..........
4a    AAA45590        EHY..A.... ..........
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FIG. 1. Sequence alignment of amino acids 192 to 211 of HCV E1 among representative genotypes. The isolates used in this study are designated by E1 clone numbers and compared with the corresponding genotypes listed in GenBank databases. A dot indicates an amino acid residue identical to that found in this study.

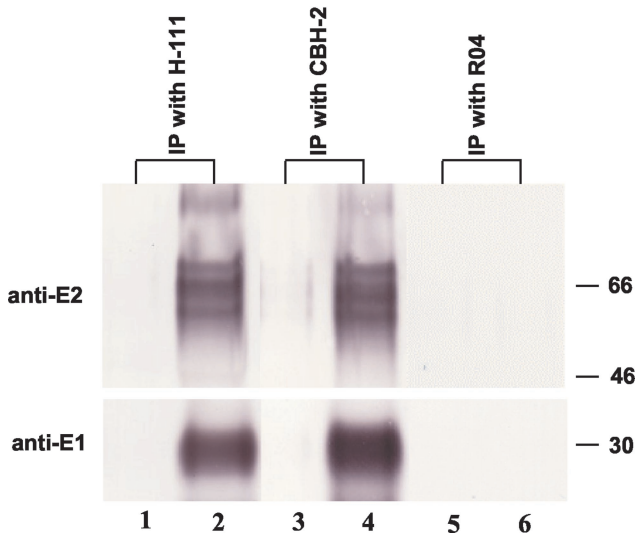

FIG. 2. H-111 immunoprecipitation (IP) of intracellularly expressed E1E2 heterodimers. HeLa cells infected with wild-type vaccinia virus vWA (lanes 1, 3, and 5) or vaccinia virus vWA plus vaccinia virus rHCV1-1488 (lanes 2, 4, and 6) were lysed 24 h postinfection and were immunoprecipitated with H-111, CBH-2 (positive control MAb to HCV E2, [5, 10]), or R04 (an isotype-matched negative control). The IP pellet was separated by sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis under reducing conditions, and immunoblots were analyzed with an anti-E2 MAb 3/11 (upper panel) and an anti-E1 MAb (Austral Biologicals) (lower panel). Sizes of proteins are shown in kilodaltons, and protein molecular markers are indicated on the right.

Since H-111 was isolated with E1 protein alone, the availability of this epitope on E1E2 heterodimers was investigated by immunoprecipitation studies (Fig. 2) with a recombinant vaccinia virus vv1488 construct (generously provided by C. Rice) containing CoreE1E2p7NS2NS3. HeLa cells infected with wild-type vaccinia virus vWA (Fig. 2, lanes 1, 3, and 5) or wild-type vaccinia virus vWA plus vaccinia virus rHCV1-1488 (lanes 2, 4, and 6) were lysed 24 h postinfection and were immunoprecipitated with H-111, CBH-2 (a conformation-dependent HMAb to HCV E2 protein [5, 10], used as a positive control), or R04, an isotype-matched negative control, as described previously (11). The precipitated proteins were separated by sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis under reducing conditions, and immunoblots were analyzed with rat anti-E2 MAb 3/11 (generously provided by J. McKeating) (8) (Fig. 2, upper panel) and the anti-E1 MAb (Austral Biologicals, San Ramon, Calif.) (Fig. 2, lower panel). Detection was performed as described previously (11). As shown in Fig. 2, H-111 was able to coprecipitate E1E2 complex from HeLa cell lysates infected with wild-type vaccinia virus plus vaccinia virus rHCV1-1488 (lane 2, upper and lower panels). Anti-HCV E2 HMAb CBH-2 (5, 10) was used to confirm the presence of the E1E2 complex in the lysates (lane 4, upper and lower panels). R04 precipitation as a negative isotype-matched HMAb (to human cytomegalovirus) control is shown in lane 6 (upper and lower panels). There were no detectable proteins in the vaccinia virus vWA (wild type)-infected lysates

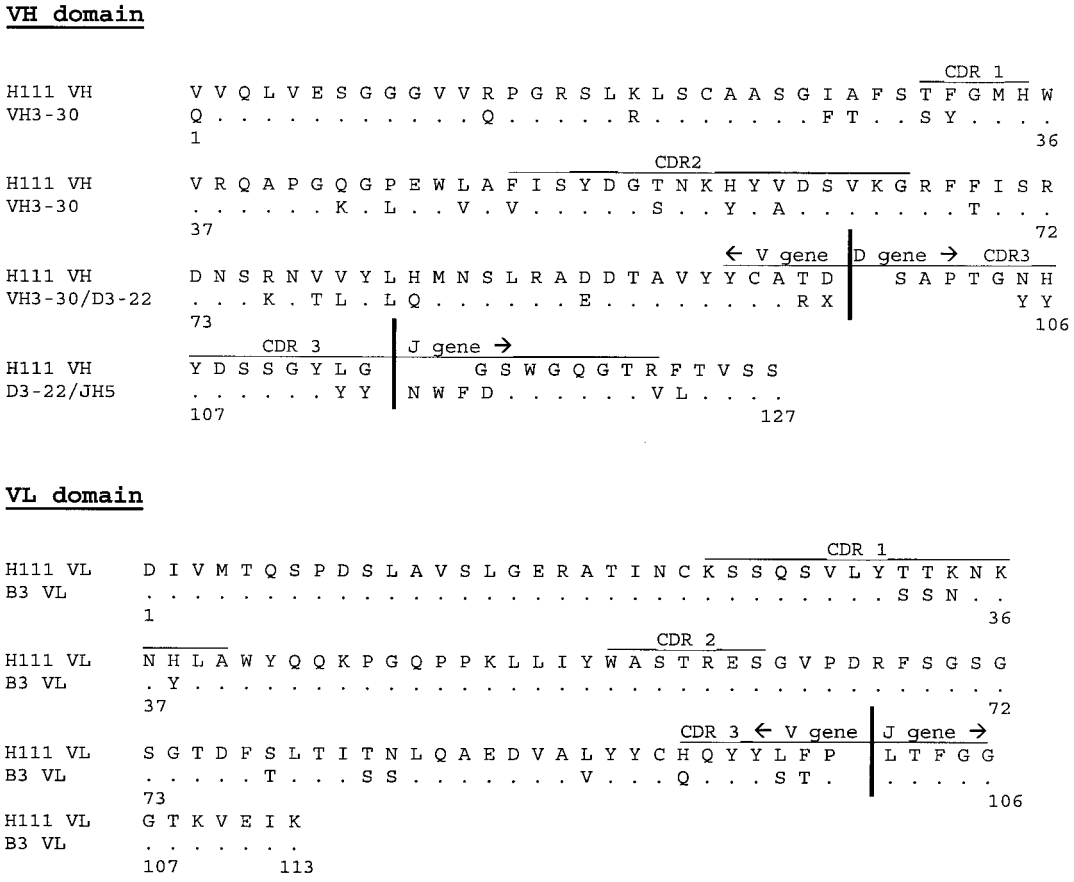

FIG. 3. H-111 V_L and V_H domain amino acid sequences translated from cDNA sequences and aligned with the corresponding germ line gene segments. A dot indicates an identical amino acid. X represents an indeterminate amino acid, as the germ line gene contains only two nucleotides at this position. CDRs are overlined, and the boundaries of the V, D, and J regions are indicated.

that were precipitated by H-111, CBH-2, or R04 (lanes 1, 3, and 5). The data suggest that H-111 recognizes an epitope that is exposed on E1E2 heterodimers.

Evidence for somatic and affinity maturation of H-111. The entire variable regions of the heavy and light chains from H-111 were sequenced to identify the framework regions (FRs) and complementarity-determining regions (CDRs), the germ line V gene counterparts, and to determine the maturational status of the V domains (Fig. 3) (GenBank accession numbers AY466500 and AY466501). The V_L and V_H domains of H-111 were amplified by RT-PCR from total cellular RNA isolated from the hybridoma cells (the RNeasy mini kit; QIAGEN) by using 5′ family-specific V leader primers and 3′ J region primers as described previously (3, 4). Sequences were analyzed by using MacVector and AssemblyLign (Oxford Molecular Group, Campbell, Calif.) and aligned with germ line sequences by using the VBASE database and DNA plot (6). As expected, the V_L and V_H domains sequenced as open reading frames, and amino acids known to be invariant were observed in the V_L domain (Cys23, Trp35, Phe62, Cys88, and Thr102) and in the V_H domain (Cys22, Thr36, and Cys92). Somatic mutations were determined by comparison with germ line genes with the highest homologies. Data bank sequences with which comparison allowed the assignment of the antibody germ line V, D, and J gene counterparts for the V_H domain were VH3-30, D3-22, and IH5, and those for the H-111 V_L domain were B3 and JK4. Extensive amino acid replacement mutations in the V gene regions of H-111 compared to the germ line V gene counterpart were evident; 32 replacements

TABLE 2. Sequence characteristics of antibody H-111 (GenBank accession numbers AY466500 and AY466501)[a]

Characteristic	V_L domain	V_H domain
CDR length (amino acids)		
CDR1	17	5
CDR2	7	17
CDR3	9	18
Germ line counterpart	B3, JK4	VH3-30, D3-22, JH5
V gene mutations (amino acids)		
Replacement	11	21
Silent	11	13
Replacement/silent, CDRs	7/4	6/3
Replacement/silent, FRs	4/7	15/10
Junctional mutations (nucleotides)		
Deletion	3	11
Insertion	0	15
Replacement	0	13

[a] V gene amino acid mutation analysis does not include V_L or V_H domain CDR3. Junctional mutations refer to nucleotide mutations at the V_L domain V-J junction and at the V_H domain V-D and D-J junctions. Corresponding amino acid mutations are shown in Fig. 3. Mutations located at 3′ end of the J gene (well beyond the D-J junction) are not included.

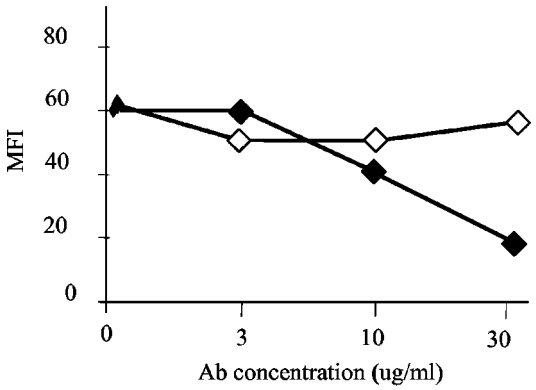

FIG. 4. H-111 inhibition of the binding of HCV-LPs to MOLT-4 cells. SYTO-labeled HCV-LPs were preincubated with increasing amounts of H-111 or control HMAb R04 and were then incubated with MOLT-4 cells. Cell-bound HCV-LPs were analyzed by flow cytometry. The *y* axis shows mean fluorescence intensity (MFI), and the *x* axis shows the antibody (Ab) concentration. Filled diamonds, H-111; open diamonds, control antibody.

were distributed as shown in Table 2. Replacement mutations were evident in the CDRs as well as the FRs, but the replacement/silent mutation ratio for H-111 CDRs (13/7) exceeded the corresponding ratio for the FRs (19/17). The mutation distribution pattern suggests that the antibody V domains of H-111 have been subjected to adaptive affinity maturational processes leading to an Ag binding specificity, as expected of an antibody isolated from peripheral B cells. Analysis of the CDR3 sequence suggested extensive junctional diversification, particularly in V_H CDR3 (Table 2). The large number of FR mutations in the V_H gene leaves open the possibility of FR involvement in antigen binding. The FRs of some antibodies have been implicated in antigen binding, either because of favorable effects on CDR folding or because of the establishment of direct contacts with the antigen (12).

H-111 blocks attachment of viruslike particles to target cells. Baculovirus-derived HCV-LPs previously shown to contain a native conformation were employed to confirm H-111 binding to native E1E2 and as a surrogate model to study virus binding (2). HCV-LPs can bind and penetrate human hepatic and lymphoid cells believed to be sites of viral replication (18). The ability of H-111 to block binding was tested by inhibiting HCV-LP entry into MOLT-4 cells (Fig. 4). The expression and purification of HCV-LPs were as described previously (17). HCV-LPs were directly labeled with the nucleic acid dye SYTO (Molecular Probes, Eugene, Oreg.) according to the manufacturer's protocol. HCV-LPs were incubated with a 5 μM concentration of SYTO in TNC buffer (10 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM $CaCl_2$) at 4°C for 15 min and repurified through a 30% sucrose cushion to remove the free dye. SYTO-labeled HCV-LPs were preincubated with increasing amounts of H-111 or isotype (control) IgG for 2 h at 4°C. The HCV-LP–antibody mixtures were incubated with MOLT-4 cells (2×10^5 cells) for 1 h. After a washing step, cell-bound HCV-LPs were analyzed directly by flow cytometry. The mean fluorescence intensity of bound HCV-LPs was determined after the subtraction of the nonspecific fluorescence value. When SYTO-labeled HCV-LPs were treated with different concentrations of H-111, HCV-LP binding to MOLT-4 cells was reduced in a dose-dependent manner, with a maximum inhibition of 70%, compared with no effect with a control antibody (Fig. 4). While non-HCV SYTO-labeled materials could account for some of the residual HCV-LP binding observed in other studies with this system (17), the lack of complete blocking by H-111 may suggest that antibodies to multiple epitopes on the surfaces of HCV-LPs are required for complete neutralization.

H-111 inhibits HCV virion infection. The recent establishment of a B-cell line (SB) from an HCV-infected non-Hodgkin's B-cell lymphoma that continuously produces infectious HCV virions in culture (16) has provided a robust means to measure neutralization of virus infectivity. Virions from SB cells can infect primary human hepatocytes, peripheral blood mononuclear cells, and a B-cell line (Raji cells) in vitro. We investigated whether the infectivity of HCV virions to Raji cells can be neutralized by H-111. The production of HCV virions, the determination of viral titers, and HCV infection assays have been described previously (16). Various concentrations of H-111 (10 to 50 μg/ml) were incubated with 0.5 ml of concentrated SB culture supernatant (HCV RNA titer, 2×10^5 IU/ml) at 4°C for 2 h before they were added to 1×10^5 Raji cells along with 0.5 ml of fresh RPMI 1640 containing 20% fetal calf serum in a 24-well plate for the infection assays (Fig. 5). An unrelated HMAb (R04) was used as an isotype-matched negative control, and interferon alpha at 1,000 U/ml was used as a positive control. After washing with prewarmed phosphate-buffered saline, total RNA was extracted from Raji cells 6 days after infection by acid-guanidinium thiocyanate-phenol-chloroform. HCV RNA in the cells was detected and quantitated by real-time RT-PCR as previously described (16). As shown in Fig. 5A, when HCV (SB) virions were preincubated with H-111 (at 10 μg/ml), the viral RNA titer in Raji cells 6 days after infection was significantly reduced compared to that of the R04 isotype-matched antibody (infection equivalent to that for no-antibody-added control). The H-111 antibody reduced viral infectivity by approximately 50%. The higher concentration of H-111 (50 μg/ml) did not further reduce infectivity (data not shown). In contrast, the isotype control antibody did not affect the virus titer. In comparison, interferon alpha at 1,000 U/ml resulted in complete reduction of the virus titer (Fig. 5B). These results suggest that H-111 mediates virus neutralization, although its efficiency was not very high. In addition to the possibility that antibodies to both E1 and E2 glycoproteins are required for complete neutralization, a second explanation is that the affinity of H-111 to genotype 2b, the genotype of this isolate (SB), is lower than that to genotype 1b. A third possibility is that some of the HCV (SB) virions are coated in part by lipoproteins that are present in fetal calf serum used to grow SB cells.

For further confirmation of the specificity of the H-111 antibody in blocking HCV infection through interaction with E1 protein, we incubated SB culture supernatant with H-111 antibody and an 14-amino-acid peptide representing the N-terminal sequence of E1 (amino acids 192 to 205) that corresponds to the H-111-binding epitope (see below) or a control peptide. The infectivity of the virus to Raji cells was then assayed. As shown in Fig. 5C, coincubation with HCV E1 peptide eliminated the inhibitory activities of H-111 whereas

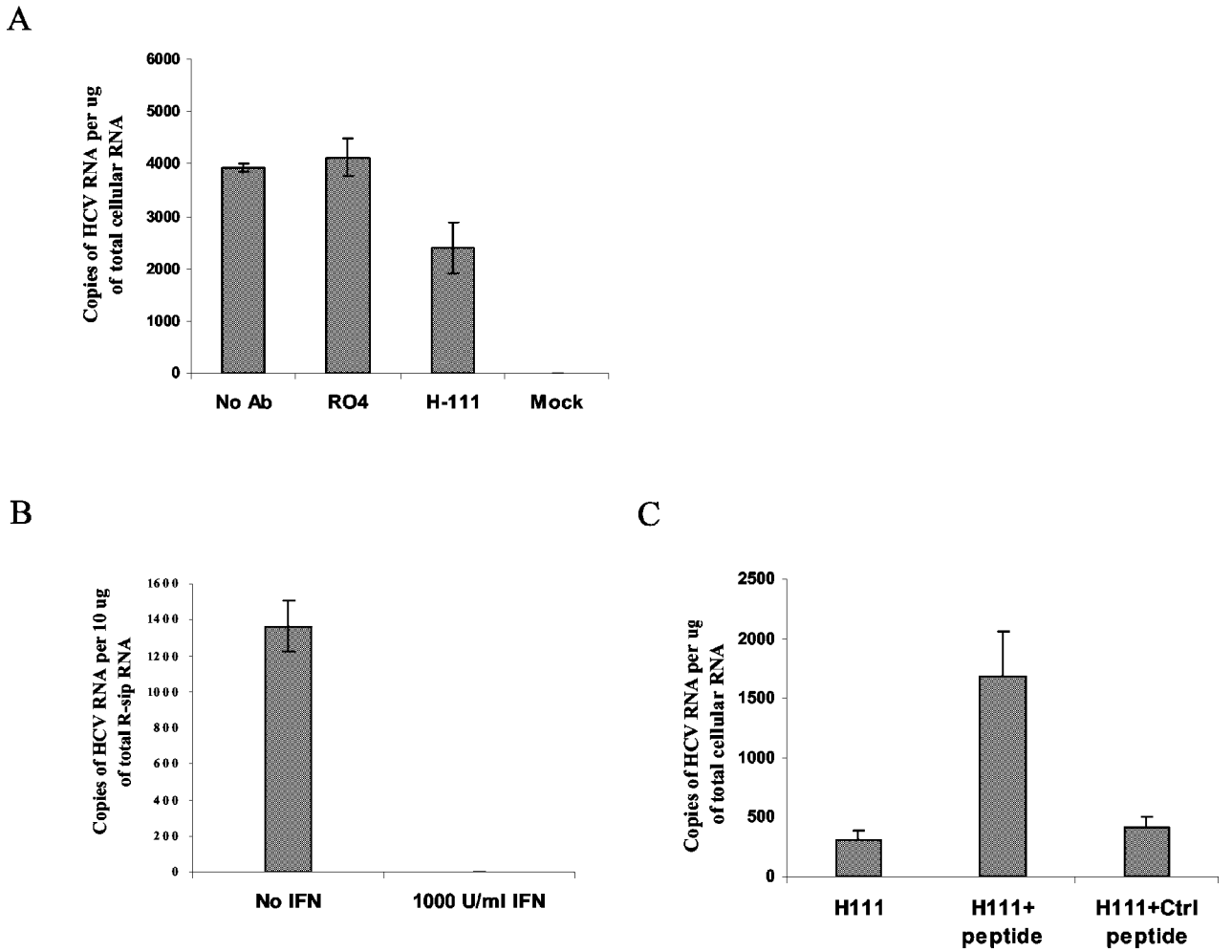

FIG. 5. H-111 neutralization of the infectivity of HCV virions. (A) HCV virions were preincubated before the infection of Raji cells with 10 μg of H-111/ml. A negative control experiment was performed with a nonspecific HMAb (R04) and no virions. Total RNA was extracted from Raji cells 6 days after infection. HCV RNA in the cells was detected and quantitated by real-time RT-PCR. (B) Alpha interferon (IFN) inhibition of HCV virion infection of Raji cells (positive control experiment). R-sip, Raji cells expressing the *sip-L* gene (19). (C) E1 peptide blocks the inhibitory activity of H-111 antibody. Preincubation with H-111 and infection was performed as described for panel A except that the incubation mixture also contained an E1 (amino acids 192 to 205) peptide or a control (Ctrl) peptide.

the control antibody did not in two separate experiments performed in triplicate. This result further suggests that H-111 antibody binds specifically to E1 and blocks HCV virus infection.

Epitope mapping of H-111. Preliminary data showed that H-111 reacted strongly with E1 by immunoprecipitation and Western blotting under reducing conditions. This result indicates that H-111 recognizes a denaturation-insensitive linear epitope within E1. To map the H-111 epitope, a series of carboxyl- and amino-terminal deletion mutants of the E1 gene were synthesized by PCR and cloned into the pDisplay vector with HA and c-*myc* tags (as described above). The E1 deletion constructs were then transfected into HEK293 cells, and the protein extracts were analyzed by ELISA and confirmed by IFA and Western blotting (data not shown) with H-111. Expression of the proteins was verified by using the HA MAb. The results obtained are summarized in Fig. 6A. Analysis of 10 carboxyl-terminal deletion E1 mutants (containing amino acids 192 to 370, 192 to 366, 192 to 352, 192 to 340, 192 to 321, 192 to 296, 192 to 269, 192 to 250, 192 to 231, and 192 to 211) showed the full retention of H-111 binding activity. For amino-terminal deletion mutants, deletion of as few as seven amino acids from the amino-terminal portion of E1 (amino acids 199 to 321) was sufficient to eliminate binding reactivity to H-111. These findings indicate that the H-111 epitope is located in the amino-terminal portion (amino acids 192 to 211) of E1. To address the possibility that the glycosyl moieties of the E1 amino terminus might be involved in the epitope, synthetic peptides representing the amino-terminal 7 and 14 amino acids of E1 were synthesized. Since the synthetic peptides are non-glycosylated, successful competition for H-111 binding would suggest that glycosylation is not required for antibody binding. E1 protein expressed by transfected HEK293 cells was captured on microtiter plates by using *Galanthus nivalis* lectin (10). Binding of H-111 was assessed in the presence of increasing amounts of the various synthetic peptides (Fig. 6B). The

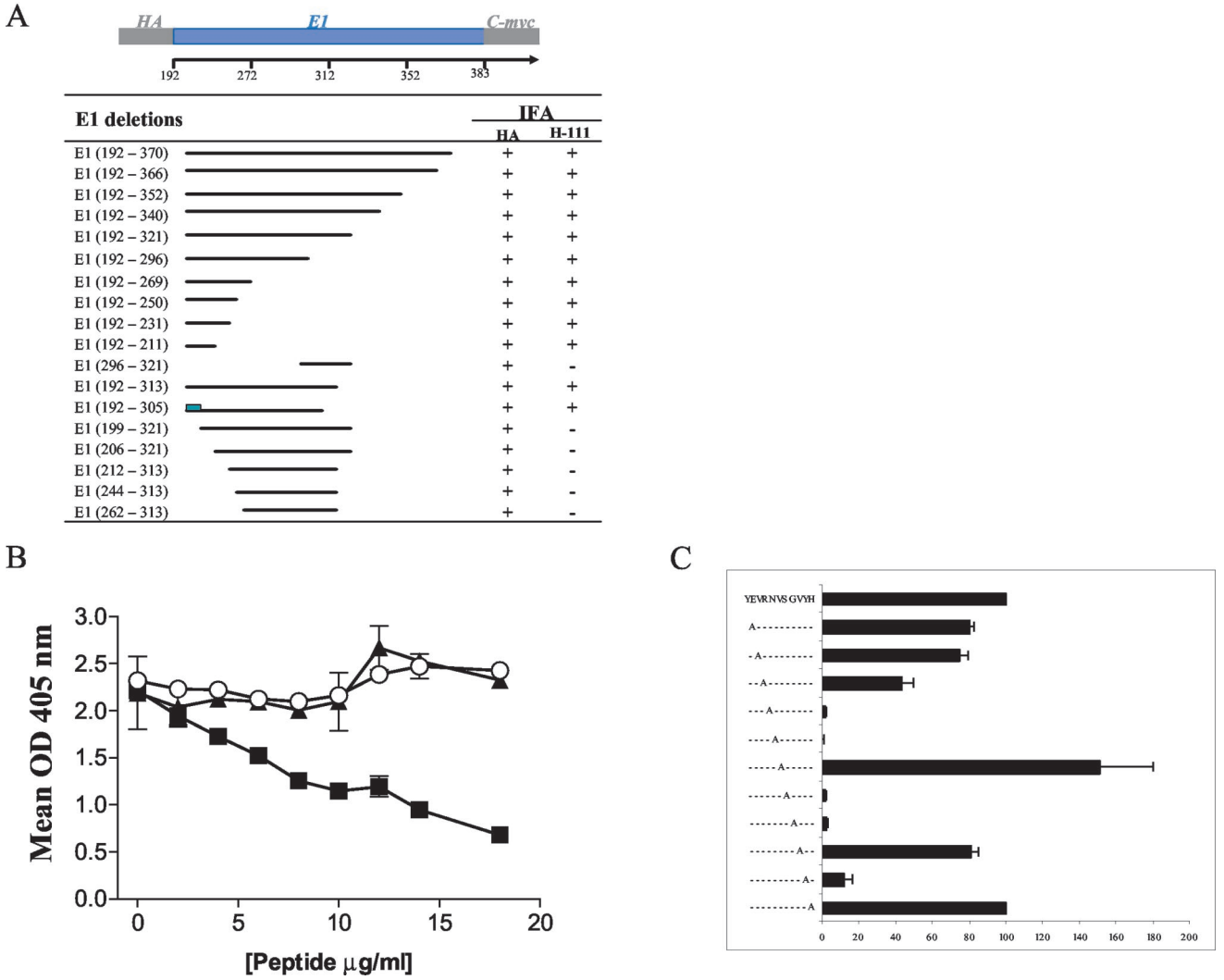

FIG. 6. H-111 epitope mapping. (A) HEK293 cells transfected with HCV E1 constructs comprising the E1 gene deletions shown were analyzed by IFA and confirmed with complete agreement by Western blotting (data not shown). The colored bar on the E1 deletion of amino acids 192 to 305 shows the specific epitope location. (B) Competition analysis of the binding of H-111 to HEK293 cells expressing E1 with overlapping synthetic peptides by ELISA. Wells were incubated with 10 μg of H-111/ml in the presence of increasing amounts (*x* axis) of blocking peptides. ○, control HTLV peptide; ▲, E1 peptide YEVRNVS; ■, E1 peptide YEVRNVSGVYHVTN. The *y* axis shows the mean optical density values for triplicate wells. Error bars represent one standard deviation from the mean. (C) Alanine scanning of H-111 epitope by ELISA. The amino acid sequence of the H-111 epitope is shown in the top row. The localization of alanine substitution is indicated in each subsequent row. The amino acid sequence of the H-111 epitope on the E1 glycoprotein is indicated on top in single-letter code. The *x* axis values are relative optical density values for binding normalized to that for the nonsubstituted sequence (top row), which was set at 100%. The value for binding to each substituted protein is the mean for three separate studies and is shown with a range bar.

addition of an irrelevant peptide from HTLV-1 gp46 had no effect on the binding of H-111 to E1, and neither did the 7-amino-acid peptide. In contrast, the addition of the 14-amino-acid peptide resulted in a dose-dependent inhibition of H-111 binding to E1. Thus, the H-111 epitope does not require glycosylation to be recognized. Alanine scanning mutagenesis was further performed to define the critical and irreplaceable amino acids within the 14-mer peptide. Mutated E1 proteins were expressed in HEK293 cells, analyzed by ELISA (Fig. 6C), and confirmed by IFA and Western blotting by using H-111. HA MAb served as a positive control, and R04 served as an isotype-matched negative control (data not shown). Amino acid substitution occurring at 195-Arg (Fig. 6C, row 5), 196-Asn (row 6), 198-Ser (row 8), and 199-Gly (row 9) eliminated H-111 binding to E1. Substitutions at 194-Val (row 4) and 201-Tyr (row 11) weakened H-111 binding to E1, supporting the idea that the central region of the 14-mer peptide is important for the interaction between H-111 and E1. Substitutions corresponding to amino acids 192-Tyr (row 2), 193-Glu (row 3), 200-Val (row 10), and 202-His (row 12) did not affect H-111 binding to E1. It is notable that 197-Val substitution enhanced binding, which may suggest a site involved in H-111 binding affinity to different genotypes and/or isolates. These results suggest that the critical amino acids in the epitope are Arg (amino acid 195), Asn (amino acid 196), Ser (amino acid 198), Gly (amino acid 199), and Tyr (amino acid 201).

The mapping of the binding or entry of this epitope-mediating virus is in agreement with a previous report (9) that described hepatocyte-binding sequences located on HCV envelope proteins by using a series of overlapping 20-mer synthetic peptides corresponding to the entire length of E1 and E2 of HCV genotype 1a. One of the hepatocyte-binding sequences in the E1 region was mapped at the N terminus (amino acids 192 to 211). In conclusion, an effective vaccine should contain conserved epitopes of different HCV genotypes that are able to elicit antibodies that block virus binding and/or entry. This report describes a candidate linear epitope containing the critical amino acids, _ _ _RN_SG_Y_, that are conserved across different HCV genotypes. This study thus establishes that E1 is required for HCV's binding, entry, and establishment of viral infection. Furthermore, this study reports the use of an HCV-producing cell line for the validation of the functional significance of one antibody, represented by H-111, as part of the immune response in HCV infection. These systems may permit further studies on the mechanism of establishment of HCV infection.

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REFERENCES

1. **Agnello, V., G. Abel, M. Elfahal, G. B. Knight, and Q.-X. Zhang.** 1999. Hepatitis C virus and other *Flaviviridae* viruses enter cells via low-density lipoprotein receptor. Proc. Natl. Acad. Sci. USA **96:**12766–12771.
2. **Baumert, T. F., S. Ito, D. T. Wong, and T. J. Liang.** 1998. Hepatitis C virus structural proteins assemble into viruslike particles in insect cells. J. Virol. **72:**3827–3836.
3. **Campbell, M. J., A. D. Zelenetz, S. Levy, and R. Levy.** 1992. Use of family specific leader region primers for PCR amplification of the human heavy chain variable region gene repertoire. Mol. Immunol. **29:**193–203.
4. **Chan, C. H., K. G. Hadlock, S. K. H. Foung, and S. Levy.** 2001. V_H1–69 gene is preferentially used by hepatitis C virus-associated B cell lymphomas and by normal B cells responding to the E2 viral antigen. Blood **97:**1023–1026.
5. **Cocquerel, L., E. R. Quinn, M. Flint, K. G. Hadlock, S. K. H. Foung, and S. Levy.** 2003. Recognition of native hepatitis C virus E1E2 heterodimers by a human monoclonal antibody. J. Virol. **77:**1604–1609.
6. **Cook, G. P., and I. M. Tomlinson.** 1995. The human immunoglobulin VH repertoire. Immunol. Today **16**:237–242.
7. **Dubuisson, J., F. Penin, and D. Moradpour.** 2002. Interaction of hepatitis C virus proteins with host cell membranes and lipids. Trends Cell Biol. **12:**517–523.
8. **Flint, M., C. Maidens, L. D. Loomis-Price, C. Shotton, J. Dubuisson, P. Monk, A. Higginbottom, S. Levy, and J. A. McKeating.** 1999. Characterization of hepatitis C virus E2 glycoprotein interaction with a putative cellular receptor, CD81. J. Virol. **73:**6235–6244.
9. **Garcia, J. E., A. Puentes, J. Suárez, R. López, R. Vera, L. E. Rodríguez, M. Ocampo, H. Curtidor, F. Guzmán, M. Urquiza, and E. Patarroyo.** 2002. Hepatitis C virus (HCV) E1 and E2 protein regions that specifically bind to HepG2 cells. J. Hepatol. **36:**254–262.
10. **Hadlock, K. G., R. E. Lanford, S. Perkins, J. Rowe, Q. Yang, S. Levy, P. Pileri, S. Abrignani, and S. K. H. Foung.** 2000. Human monoclonal antibodies that inhibit binding of hepatitis C virus E2 protein to CD81 and recognize conserved conformational epitopes. J. Virol. **74:**10407–10416.
11. **Hadlock, K. G., J. Rowe, S. Perkins, P. Bradshaw, G.-Y. Song, C. Cheng, J. Yang, R. Gascon, J. Halmos, S.-M. Rehman, M. McGrath, and S. K. H. Foung.** 1997. Neutralizing human monoclonal antibodies to conformational epitopes of human T-cell lymphotropic virus type 1 and 2 gp46. J. Virol. **71:**5828–5840.
12. **Li, Y., M. R. Owen, W. Cockburn, I. Kumagai, and G. C. Whitelam.** 1996. Study of antibody-antigen interaction through site-directed mutagenesis of the VH region of a hybrid phage-antibody fragment. Protein Eng. **12**:1211–1217.
13. **Major, M. E., B. Rehermann, and S. M. Feinstone.** 2001. Hepatitis C viruses, p. 1127–1162. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology. Lippincott Williams & Wilkins, Philadelphia, Pa.
14. **Monazahian, M., I. Bohme, S. Bonk, A. Kock, C. Scholz, S. Grete, and R. Thomssen.** 1999. Low-density lipoprotein receptor as a candidate receptor for hepatitis C virus. J. Med. Virol. **57:**223–229.
15. **Perkins, S., and S. K. H. Foung.** 1995. Stabilizing antibody secretion of human Epstein-Barr virus-activated B-lymphocytes with hybridoma formation by electrofusion. Methods Mol. Biol. **48:**295–307.
16. **Sung, V. M.-H., S. Shimodaira, A. L. Doughty, G. R. Picchio, H. Can, T. S. B. Yen, K. L. Lindsay, A. M. Levine, and M. M. C. Lai.** 2003. Establishment of B-cell lymphoma cell lines persistently infected with hepatitis C virus in vivo and in vitro: the apoptotic effects of virus infection. J. Virol. **77:**2134–2146.
17. **Triyatni, M., B. Saunier, P. Maruvada, A. R. Davis, L. Ulianich, T. Heller, A. Patel, L. D. Kohn, and T. J. Liang.** 2002. Interaction of Hepatitis C virus-like particles and cells: a model system for studying viral binding and entry. J. Virol. **76:**9335–9344.
18. **Triyatni, M., J. Vergalla, A. R. Davis, K. G. Hadlock, S. K. H. Foung, and T. J. Liang.** 2002. Structural features of envelope proteins of hepatitis C virus-like particles as determined by anti-envelope monoclonal antibodies and CD81 binding. Virology **298:**124–132.
19. **Yeh, C.-T., H.-Y. Lai, T.-C. Chen, C.-M. Chu, and Y.-F. Liaw.** 2001. Identification of a hepatic factor capable of supporting hepatitis C virus replication in a nonpermissive cell line. J. Virol. **75**:11017–11024.